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SEARCH REQUEST FORM

Examiner # (Mandatory): Lisa V. Cook Requester's Full Name: Lisa V. Cook

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Serial Number: 09/419,901 Results Format Preferred (circle): PAPER DISK E-MAIL

Title of Invention Methods of Diagnosing Mucosal Damage

Inventors (please provide full names): Jennifer E. Von E/K, Ralf Lahlugger, Irena Neverova

Earliest Priority Date: 10/18/98

Keywords (include any known synonyms registry numbers, explanation of initialisms):

Please see attached claims + data sheet.

Thanks, Lisa

Search Topic:

Please write detailed statement of the search topic, and the concept of the invention. Describe as specifically as possible the subject matter to be searched. Define any terms that may have a special meaning. Give examples of relevant citations, authors, etc., if known. You may include a copy of the abstract and the broadcast or most relevant claim(s).

Point of Contact:
Mary Hale
Technical Info. Specialist
CM1 12D16 Tel: 308-4258

821
801

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PTO-1590 (2-99)

L. Cook
419901

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=> fil reg;e myofilament protein/cn

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E1            1     MYOFER/CN
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E6            1     MYOFLEXINE/CN
E7            1     MYOGEN (CARP MUSCLE)/CN
E8            1     MYOGENIN (CARP)/CN
E9            1     MYOGENIN (CHICKEN MUSCLE PROTEIN MOIETY REDUCED)/CN
E10           1     MYOGENIN (CYPRINUS CARPIO)/CN
E11           1     MYOGENIN (HUMAN GENE MYF4 REDUCED)/CN
E12           1     MYOGENIN (MOUSE C2 CELL REDUCED)/CN

=> s (alkaline phosphatase or horseradish peroxide or luciferase or
"beta-galactosidase" or lysozyme or "glucose-6-phosphate dehydrogenase" or
lactate dehydrogenase or urease)/cn

              1 ALKALINE PHOSPHATASE/CN
              0 HORSERADISH PEROXIDE/CN
              6 LUCIFERASE/CN
              0 "BETA-GALACTOSIDASE"/CN
              1 LYSOZYME/CN
              0 "GLUCOSE-6-PHOSPHATE DEHYDROGENASE"/CN
              1 LACTATE DEHYDROGENASE/CN
              1 UREASE/CN
L1           10 (ALKALINE PHOSPHATASE OR HORSERADISH PEROXIDE OR LUCIFERASE OR
                "BETA-GALACTOSIDASE" OR LYSOZYME OR "GLUCOSE-6-PHOSPHATE
DEHYDRO
                GENASE" OR LACTATE DEHYDROGENASE OR UREASE)/CN

=> s (troponin i or troponin t or troponin c or "alpha-actinin" or actin or
tropomyosin or desmin or myosin light chain 1 or myosin light chain 2 or
myosin light chain 3)/cn

              1 TROPONIN I/CN
              0 TROPONIN T/CN
              0 TROPONIN C/CN
              0 "ALPHA-ACTININ"/CN
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             1 ACTIN/CN
             0 TROPOMYOSIN/CN
             0 DESMIN/CN
             0 MYOSIN LIGHT CHAIN 1/CN
             0 MYOSIN LIGHT CHAIN 2/CN
             0 MYOSIN LIGHT CHAIN 3/CN
L2           2 (TROPONIN I OR TROPONIN T OR TROPONIN C OR "ALPHA-ACTININ" OR
               ACTIN OR TROPOMYOSIN OR DESMIN OR MYOSIN LIGHT CHAIN 1 OR
MYOSIN
                LIGHT CHAIN 2 OR MYOSIN LIGHT CHAIN 3)/CN

=> fil medl,caplus,biosis,embase,wpids

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=> s (alkaline phosphatase or horseradish peroxide or luciferase or
"beta-galactosidase" or lysozyme or "glucose-6-phosphate dehydrogenase" or
lactate dehydrogenase or urease or l1) and muscle damag?

L3           71 FILE MEDLINE
L4           84 FILE CAPLUS
L5           78 FILE BIOSIS
L6           64 FILE EMBASE
L7            1 FILE WPIDS

TOTAL FOR ALL FILES
L8          298 (ALKALINE PHOSPHATASE OR HORSERADISH PEROXIDE OR LUCIFERASE OR
                "BETA-GALACTOSIDASE" OR LYSOZYME OR "GLUCOSE-6-PHOSPHATE
DEHYDRO
                GENASE" OR LACTATE DEHYDROGENASE OR UREASE OR L1) AND MUSCLE
                DAMAG?

=> s l8 and (troponin i or troponin t or troponin c or "alpha-actinin" or
actin or tropomyosin or desmin or myosin light chain 1 or myosin light chain
2 or myosin light chain 3 or l2 or myofilament protein modif?)

L9            3 FILE MEDLINE
L10           2 FILE CAPLUS
L11           1 FILE BIOSIS
L12           5 FILE EMBASE
L13           0 FILE WPIDS

TOTAL FOR ALL FILES
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L14 11 L8 AND (TROPONIN I OR TROPONIN T OR TROPONIN C OR "ALPHA-ACTININ
" OR ACTIN OR TROPOMYOSIN OR DESMIN OR MYOSIN LIGHT CHAIN 1 OR MYOSIN LIGHT CHAIN 2 OR MYOSIN LIGHT CHAIN 3 OR L2 OR MYOFILAMEN
T PROTEIN MODIF?)

=> dup rem l14

PROCESSING COMPLETED FOR L14
L15 7 DUP REM L14 (4 DUPLICATES REMOVED)

=> d 1-7 cbib abs

Applicant

L15 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2000 ACS
1999:444526 Document No. 131:56156 Methods of diagnosing **muscle damage** by studying **myofilament protein modification** products. Van Eyk, Jennifer E.; Iscoe, Steven D.; Simpson, Jeremy A. (Queen's University at Kingston, Can.). Can. Pat. Appl. CA 2243372 AA 19990116, 77 pp. (English). CODEN: CPXXEB. APPLICATION: CA 1998-2243372 19980716. PRIORITY: US 1997-52697 19970716; US 1998-115589 19980715.

AB A method for assessing **muscle damage** in a biol. sample obtained from a subject is disclosed. The method involves obtaining a biol. sample from a subject being assessed for **muscle damage**, and evaluating the sample for the presence or absence of a **myofilament protein modification** product. The method can also be used to assess the extent and/or type of **muscle damage** in a subject by studying the profile of **myofilament protein modification** products detected in the sample taken from the subject. The invention further provides a method for screening for an agent which modulates the level of a **myofilament protein modification** product present in a biol. sample or for a calcium sensitizing agent. The invention is applicable to cardiac muscle and skeletal muscle. SDS-PAGE and Western blot anal. of rat cardiac muscle tissue samples indicated that the extent and type of modification to **troponin I** changed depending on whether mild or severe ischemic damage had occurred.

L15 ANSWER 2 OF 7 MEDLINE
97479502 Document Number: 97479502. Skeletal **troponin I** as a marker of exercise-induced **muscle damage**.
Sorichter S; Mair J; Koller A; Gebert W; Rama D; Calzolari C; Artner-Dworzak E; Puschendorf B. (Department of Medical Chemistry and Biochemistry and of, University of Innsbruck Medical School, A-6020 Innsbruck, Austria.)JOURNAL OF APPLIED PHYSIOLOGY, (1997 Oct) 83 (4) 1076-82. Journal code: HEG. ISSN: 8750-7587. Pub. country: United States.
Language: English.

AB The utility of skeletal **troponin I** (sTnI) as a plasma marker of skeletal **muscle damage** after exercise was compared against creatine kinase (CK), myoglobin (Mb), and myosin heavy chain (MHC) fragments. These markers were serially measured in normal physical education teacher trainees after four different exercise regimens: 20 min of level or downhill (16% decline) running (intensity: 70% maximal O2 uptake), high-force eccentric contractions (70 repetitions), or high-force isokinetic concentric contractions of the quadriceps group (40 repetitions). Eccentrically biased exercise (downhill running and eccentric contractions) promoted greater increases in all

parameters. The highest plasma concentration were found after downhill running {median peaks: 309 U/l CK concentration (-CK-)}, 466 microg/l Mb concentration (-Mb-), 1,021 microU/l MHC concentration (-MHC-), and 27.3 microg/l sTnI concentration ([sTnI]). Level running produced a moderate response (median peaks: 178 U/l -CK-, 98 microg/l -Mb-, 501 microU/l -MHC-, and 6.6 microg/l [sTnI]), whereas the concentric contraction protocol did not elicit significant changes in any of the markers assayed.

sTnI increased and peaked in parallel to CK and stayed elevated (>2.2 microg/l) for at least 1-2 days after exercise. In contrast to MHC, sTnI is an initial, specific marker of exercise-induced muscle injury, which may be partly explained by their different intracellular compartmentation with essentially no (MHC <0.1%) or a small soluble pool (sTnI: median 3.4%).

L15 ANSWER 3 OF 7 MEDLINE DUPLICATE 1

97207799 Document Number: 97207799. Progress in myocardial damage detection:

new biochemical markers for clinicians. Mair J. (Institut fur Medizinische

Chemie and Biochemie, University of Innsbruck, Austria.. Johannes.Mair@uibk.ac.at). CRITICAL REVIEWS IN CLINICAL LABORATORY SCIENCES, (1997) 34 (1) 1-66. Ref: 174. Journal code: AFY. ISSN: 1040-8363. Pub. country: United States. Language: English.

AB New clinical requirements for triaging chest pain patients challenge the abilities of the current cardiac markers. Serial measurements of myoglobin, creatine kinase (CK) isoenzyme MB (CKMB) mass, or CK isoforms in emergency rooms help to rapidly rule out acute myocardial infarction (AMI). However, within the first 3 to 4 h from chest pain onset, their sensitivities are too low to contribute significantly to AMI diagnosis during this period. CKMB and **lactate dehydrogenase** (LDH) isoenzyme 1 are not heart-specific, which hampers reliable diagnosis in patients with concomitant skeletal **muscle damage**. By contrast, the regulatory proteins **troponin I** and **troponin T** are expressed in three different isoforms: one for slow-twitch skeletal muscle fibers, one for fast-twitch skeletal muscle fibers, and one for cardiac muscle (cTnI, cTnT); cardiac-specific cTnI and cTnT assays are already available for routine use. cTnT and cTnI are the most promising markers for risk stratification in patients with unstable angina pectoris. Recent reports on increased cTnT in patients with renal failure or myopathy without evidence of myocardial injury and undetectable cTnI suggest that cTnT could be reexpressed similar to CKMB and LDH-1 in chronically damaged human skeletal muscle. Therefore, cTnI is probably the most heart-specific marker. Among the recently proposed new markers for early AMI diagnosis: glycogen phosphorylase isoenzyme BB (GPBB), fatty acid binding protein, phosphoglyceric acid mutase isoenzyme MB, enolase isoenzyme alpha beta, S100a0, and annexin V, GPBB is the most promising because it increases as early as 1 to 4 h from chest pain onset and its early release appears to be essentially dependent on ischemic myocardial injury.

L15 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 2

1996:517516 Document No. 125:324085 Serum cardiac **troponin T** after repeated endurance exercise events. Bonetti, A.; Tirelli, F.; Albertini, R.; Monica, C.; Monica, M.; Tredici, G. (Institute Medical Pathology, University Parma, Italy). Int. J. Sports Med., 17(4), 259-262 (English) 1996. CODEN: IJSMDA. ISSN: 0172-4622.

AB Recently Dr. Rowe made a hypothesis according to which small areas of myocardial necrosis can be caused by microvascular spasm, related to high

catecholamine concns. and other mechanisms, following extraordinary unremitting endurance exercises or due to the cumulative effect of several endurance events. It was this last suggestion which prompted us to investigate 25 top cyclists, taking part in the 77th Giro d'Italia. Blood samples were obtained the day before the start of the competition and once a week thereafter until the end. We measured myoglobin, lactic dehydrogenase, total creatine kinase, creatine kinase isoenzyme MB and serum cardiac **troponin T** (Tn-T), a highly sensitive and specific method for the detection of myocardial injury. While at measuring time points which followed we found a significant increase in the serum indicators of **muscle damage**, compared with their values at the beginning of the race, creatine kinase isoenzyme MB did not rise significantly and cardiac Tn-T was found in the serum of only 5 athletes, repeatedly in some cases, but always below the cut off values considered as indicating myocardial ischemia. On the basis of the behavior of creatine kinase isoenzyme MB and, above all, of cardiac Tn-T, we can conclude that heavy endurance exercises, repeated daily for 22 days, as was the case in our study, do not seem able to produce, in top athletes, permanent heart damage by means of acute myocardial injury.

L15 ANSWER 5 OF 7 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
94381123 EMBASE Document No.: 1994381123. Beyond CK-MB: Biochemical markers for perioperative myocardial infarction. Mangano D.T.. Anesthesiology Service, Veterans Affairs Medical Center, University of California, 4150 Clement Street,San Francisco, CA 94121, United States. Anesthesiology 81/6 (1317-1320) 1994.
ISSN: 0003-3022. CODEN: ANESAV. Pub. Country: United States. Language: English. Summary Language: English.
AB Diagnosis of perioperative myocardial infarction remains an important but challenging task. Both clinical symptoms and electrocardiographic changes have inherent limitations. Therefore, biochemical markers for myocardial injury are critical diagnostic tools. The use of creatine kinase isoenzymes (CK-MB) has enhanced detection of perioperative myocardial infarction; however, skeletal **muscle damage** during surgery limits CK-MB specificity. In this regard, the cardiac troponins appear to offer increased sensitivity, primarily because of their prolonged diagnostic window and even may offer enhanced specificity (especially **troponin-I**) in patients with surgical skeletal **muscle damage**. In addition, the convenience of relatively infrequent sampling (because of the prolonged diagnostic window), as well as potential cost savings, make use of the troponin markers attractive. However, definitive data in high-risk patients undergoing either cardiac or noncardiac surgery are still lacking, and significant questions remain regarding appropriate thresholds, specificity of **troponin-T**, and comparative accuracy of **troponin-T**, **troponin-I**, and CK-MB for diagnosis (and prognosis) of perioperative myocardial infarction.

L15 ANSWER 6 OF 7 MEDLINE DUPLICATE 3
93186262 Document Number: 93186262. Circulating alpha-**actin** protein in acute myocardial infarction. Aranega A E; Reina A; Muros M A; Alvarez L; Prados J; Aranega A. (Department of Morphological Sciences, School of Medicine, University of Granada, Spain..)INTERNATIONAL JOURNAL OF CARDIOLOGY, (1993 Jan) 38 (1) 49-55. Journal code: GQW. ISSN: 0167-5273. Pub. country: Netherlands. Language: English.
AB We used Western-blot analysis to investigate the possible presence in the

bloodstream of the contractile protein alpha-**actin** in 70 patients diagnosed with acute myocardial infarction on the basis of clinical, electrocardiographic and laboratory (creatine kinase and **lactate dehydrogenase**) criteria. Circulating protein was identified with a monoclonal antibody specific for cardiac alpha-**actin**. Of the 70 control samples of blood, the immunoblot results were negative for alpha-**actin** in 98% of the cases. Of the 30 patients with skeletal **muscle damage** caused by surgery, 26 were negative for circulating alpha-**actin**. Of the 70 patients with acute myocardial infarction, circulating alpha-**actin** was found in 67 (95%) as a 43 kDa band in immunoblots; the highest circulating concentrations (0.0580 micrograms/microliters) were found in those with anterior acute myocardial infarction. Circulating alpha-**actin** was detected in samples taken between 1 and 180 h after the onset of pain, and showed a biphasic pattern of appearance. Our findings for serum alpha-**actin**, together with the relationship between serum concentrations of this protein and sex ($p = 0.001$), tobacco use ($p = 0.007$) and postepisode complications ($p = 0.002$), should make it possible to gain a deeper understanding of acute myocardial infarction as a clinical entity.

L15 ANSWER 7 OF 7 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

91127387 EMBASE Document No.: 1991127387. Diagnostic efficiency of **troponin T** measurements in acute myocardial infarction.

Katus H.A.; Remppis A.; Neumann F.J.; Scheffold T.; Diederich K.W.; Vinar G.; Noe A.; Matern G.; Kuebler W.. Innere Medizin INFARCTION, Medizinische Univ.-Klinik, Bergheimer Strasse 58,6900 Heidelberg, Germany. Circulation 83/3 (902-912) 1991.

ISSN: 0009-7322. CODEN: CIRCAZ. Pub. Country: United States. Language: English. Summary Language: English.

AB The present study was designed to evaluate the efficiency of a newly developed **troponin T** enzyme immunoassay for the detection of acute myocardial infarction. The study comprised 388 patients admitted with chest pain and suspected myocardial infarction and 101 patients with skeletal **muscle damage** and additional suspected myocardial cell damage. **Troponin T** was elevated to more than twice the analytical sensitivity of the assay (0.5 .mu.g/l) in all patients with non-Q wave (range, 1.2-5 .mu.g/l) and Q wave infarction (range, 3-220 .mu.g/l). **Troponin T** appeared in serum as early as 3 hours after onset of pain in 50% of the patients and remained elevated in all patients for more than 130 hours, revelaing release kinetics of both free cytosolic and structurally bound molecules. The diagnostic efficiency of **troponin T** was superior to that of creatine kinase-MB (98% versus 97%) and remained at 98% until 5.5 days after admission, if patients with unstable angina were excluded from analysis. In the 79 patients with unstable an gina, **troponin T** was elevated (range, 0.55-3.1 .mu.g/l) in at least one blood sample from each of 37 patients (56%). Circulating **troponin T** was correlated to the presence of reversible ST segment or T wave changes on the electrocardiogram ($p < 0.005$) and to the frequency of in-hospital complications. In the 101 patients with skeletal **muscle damage** and suspected additional cardiac **muscle damage**, **troponin T** was the most useful test; its efficiency was 89% or 94% (depending on the discriminator value used) as compared with 63% for creatine kinase-MB. Thus, the data of the study indicate that the newly developed **troponin T** test improves the efficiency of

serodiagnostic tools for the detection of myocardial cell necrosis as compared with conventionally used cardiac enzymes.

=> s (alkaline phosphatase or horseradish peroxide or luciferase or "beta-galactosidase" or lysozyme or "glucose-6-phosphate dehydrogenase" or lactate dehydrogenase or urease or l1) and (hypox? or ischem? or reperfus?)

L16 2697 FILE MEDLINE
L17 2606 FILE CAPLUS
L18 2538 FILE BIOSIS
L19 2467 FILE EMBASE
L20 34 FILE WPIDS

TOTAL FOR ALL FILES
L21 10342 (ALKALINE PHOSPHATASE OR HORSERADISH PEROXIDE OR LUCIFERASE OR "BETA-GALACTOSIDASE" OR LYSOZYME OR "GLUCOSE-6-PHOSPHATE DEHYDRO
GENASE" OR LACTATE DEHYDROGENASE OR UREASE OR L1) AND (HYPOX? OR ISCHEM? OR REPERFUS?)

=> s l21 and (troponin i or troponin t or troponin c or "alpha-actinin" or actin or tropomyosin or desmin or myosin light chain 1 or myosin light chain 2 or myosin light chain 3 or l2 or myofilament protein modif?)

L22 34 FILE MEDLINE
L23 33 FILE CAPLUS
L24 33 FILE BIOSIS
L25 45 FILE EMBASE
L26 1 FILE WPIDS

TOTAL FOR ALL FILES
L27 146 L21 AND (TROPONIN I OR TROPONIN T OR TROPONIN C OR "ALPHA-ACTINI
N" OR ACTIN OR TROPOMYOSIN OR DESMIN OR MYOSIN LIGHT CHAIN 1 OR
MYOSIN LIGHT CHAIN 2 OR MYOSIN LIGHT CHAIN 3 OR L2 OR MYOFILAMEN
T PROTEIN MODIF?)

=> s (phosphorylat? or glycosylat? or myristylat? or phenylat? or acetylat? or nitrosylat? or sulphation or sulfation) and l27

L28 0 FILE MEDLINE
L29 1 FILE CAPLUS
L30 0 FILE BIOSIS
L31 0 FILE EMBASE
L32 0 FILE WPIDS

TOTAL FOR ALL FILES
L33 1 (PHOSPHORYLAT? OR GLYCOSYLAT? OR MYRISTYLAT? OR PHENYLAT? OR ACETYLAT? OR NITROSYLAT? OR SULPHATION OR SULFATION) AND L27

=> d cbib abs

L33 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2000 ACS
1999:701153 **Hypoxic** response of synaptosomal proteins in term guinea pig fetuses. Buonocore, Giuseppe; Liberatori, Sabrina; Bini, Luca; Mishra, Om P.; Delivoria-Papadopoulos, Maria; Pallini, Vitaliano; Bracci, Rodolfo (Institute of Preventive Pediatrics and Neonatology, University of

Siena, Siena, 53100, Italy). J. Neurochem., 73(5), 2139-2148 (English) 1999. CODEN: JONRA9. ISSN: 0022-3042. Publisher: Lippincott Williams & Wilkins.

AB Early events in the **hypoxia**-induced response trigger tyrosine **phosphorylation** cascades involving a large no. of enzymes and substrates. The resolving power of advanced two-dimensional gel electrophoresis, followed by immunoblotting with specific antibodies to phosphotyrosine, has been used to analyze **hypoxia**-induced modifications in guinea pig brain synaptosomes. These procedures, in conjunction with computer-aided image anal., are useful in the differential display of gene products, providing comparison at the level of post-translationally modified products. Studies were performed in cerebral cortical synaptosomes from three normoxic and three **hypoxic** newborn guinea pigs. To filter off background noise consisting of non-reproducible migrating protein spots, only reproducible features of electrophoretic patterns were considered. Immunoreactivity patterns obtained with anti-phosphotyrosine antibodies proved to be different in normoxic and **hypoxic** synaptosomes: of a total of 130 immunoreactive spots, 49 were tyrosine-**phosphorylated** in **hypoxic** synaptosomes only and 20 in the normoxic ones only. Our data suggest that **hypoxia** extensively remodels the signaling pathway by switching off tyrosine **phosphorylation** of some cellular components (i.e., .alpha.-internexin) and switching on tyrosine **phosphorylation** of some other proteins (i.e., heat shock cognate 70, aconitase, 2',3'-cyclic nucleotide 3'-phosphodiesterase, and pyruvate kinase).

```
=> s l27 and (elisa or hplc or assay)

L34           7 FILE MEDLINE
L35           9 FILE CAPLUS
L36           7 FILE BIOSIS
L37          12 FILE EMBASE
L38           0 FILE WPIDS

TOTAL FOR ALL FILES
L39          35 L27 AND (ELISA OR HPLC OR ASSAY)

=> s l39 not (l33 or l14)

L40           6 FILE MEDLINE
L41           8 FILE CAPLUS
L42           7 FILE BIOSIS
L43          11 FILE EMBASE
L44           0 FILE WPIDS

TOTAL FOR ALL FILES
L45          32 L39 NOT (L33 OR L14)

=> dup rem l45

PROCESSING COMPLETED FOR L45
L46          14 DUP REM L45 (18 DUPLICATES REMOVED)

=> d cbib abs 1-14


L46  ANSWER 1 OF 14  EMBASE  COPYRIGHT 2000 ELSEVIER SCI. B.V.
1999332280  EMBASE  [The efficiency of troponin determination in cardiac
     diagnosis]. LEISTUNGSFAHIGKEIT DER TROPONINBESTIMMUNG IN DER KARDIALEN
```

DIAGNOSTIK. Mair J.. Dr. J. Mair, Universitatsklin. fur Innere Medizin, Klinische Abteilung fur Kardiologie, Anichstrasse 35, A-6020 Innsbruck, Austria. Johannes.Mair@uibk.ac.at. Journal fur Kardiologie 6/9 (463-474) 1999.
Refs: 34.
ISSN: 1024-0098. CODEN: JKARFN. Pub. Country: Austria. Language: German. Summary Language: English; German.

AB The development of commercially available **assays** for the determination of cardiac **troponin I** (TnI) and **troponin T** (TnT) was the most important innovation in the field of cardiovascular laboratory diagnostics during recent years, it was a diagnostic breakthrough in routine diagnosis. Troponins are the new criterion standard and should be offered by all hospital-based laboratories. Due to their superior specificities troponins are the markers of choice for the diagnosis of acute myocardial damage in presence or the possibility of concomitant skeletal muscle injury, e g. diagnosis of perioperative myocardial infarctions or myocardial infarctions after resuscitation. Based on their high sensitivities troponins make possible new clinical applications of cardiac markers. For example, in approximately 30 % of patients with unstable angina at rest cardiac troponins are detectable in peripheral-venous blood samples without increases in creatine kinase (CK) or its isoenzyme MB (CKMB). TnI and TnT are from ECG- findings independent risk factors for short-term as well as long-term prognosis in patients with acute coronary syndromes. Compared with other diagnostic procedures with similar diagnostic and prognostic information troponin determination is cheap, and the results are easy to interpret. Recent retrospective troponin measurements in blood samples of therapeutic trials suggest that in the future patients with acute coronary syndromes are likely to be treated differently depending on their troponin concentrations. After implementation of troponin determination in the laboratory it is necessary to delete old cardiac markers (CKMB, **lactate dehydrogenase**, aspartate-aminotransferase) from the cardiac routine panel to increase cost- effectiveness.

L46 ANSWER 2 OF 14 MEDLINE DUPLICATE 1
1999377720 Document Number: 99377720. Impact of troponins on the evaluation and treatment of patients with acute coronary syndromes. Adams J 3rd. (Jewish Heart and Lung Institute, Jewish Hospital, Louisville, KY 40202, USA.. jadams03@sprynet.com). CURRENT OPINION IN CARDIOLOGY, (1999 Jul) 14 (4) 310-3. Ref: 18. Journal code: BDA. ISSN: 0268-4705. Pub. country: United States. Language: English.

AB Cardiac troponins possess superior sensitivity and specificity for the detection of cardiac injury. They can be used successfully to replace measurements of MB isoenzyme of creatine kinase or **lactate dehydrogenase** for the retrospective diagnosis of myocardial infarction. Measurement of these proteins confers powerful prognostic information that can be used to triage patients. An increasing body of data suggests that measurement of troponin proteins can be useful to guide therapeutic decisions in patients with acute coronary artery syndromes, especially regarding treatment with low-molecular-weight heparin or IIB/IIIA inhibitors. The absence of troponins in the circulation does not necessarily indicate the absence of coronary artery disease. With current **assays**, a significant diagnostic difference does not appear to exist between cardiac **troponin I** and T in patients with acute coronary artery syndromes.

L46 ANSWER 3 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
1999211422 EMBASE Biochemical markers of cardiac damage: From traditional enzymes to cardiac-specific proteins. Wu A.H.B.. Dr. A.H.B. Wu, Department of Pathology, Hartford Hospital, 80 Seymour St., Hartford, CT 06102, United States. awu@harthosp.org. Scandinavian Journal of Clinical and Laboratory Investigation, Supplement 59/230 (74-82) 1999.
Refs: 44.
ISSN: 0085-591X. CODEN: SCLSAH. Pub. Country: Norway. Language: English. Summary Language: English.

AB Measurement of cardiac markers in blood has been the mainstay for diagnosis of acute myocardial infarction for nearly 50 years. The field has evolved from measurement of enzyme activity to mass concentrations of proteins using automated non-isotopic immunoassays. With changing clinical practices, cardiac markers are now needed to detect the presence of minor myocardial infarction in patients with unstable angina. Outcome studies have shown that patients with increased troponin are at high short-term risk for death and AMI. Recent developments involve the use of cardiac markers to select the most appropriate therapy for patients with acute coronary syndromes. The success of new cardiac markers such as troponin is due to their high cardiac specificity and the existence of **assays** with low detection limits. Traditional enzymes such as CK and CK-MB are thought to be released only in situations of irreversible myocardial necrosis. In the case of cardiac troponin, clinical observations and animal studies suggest that cytosolic free troponin may be released in reversible **ischemia** in addition to irreversible cell damage. The IFCC S-SCM has recommended use of two cut-off concentrations for cardiac troponin to differentiate normal from minor myocardial injury and AMI. A low cut-off may detect reversible **ischemic** events in some cases.

L46 ANSWER 4 OF 14 MEDLINE DUPLICATE 2
1999331616 Document Number: 99331616. Time-course of cardiac **troponin I** release from isolated perfused rat hearts during **hypoxia**/reoxygenation and **ischemia**/**reperfusion**. Bertinchant J P; Polge A; Robert E; Sabbah N; Fabbro-Peray P; Poirey S; Laprade M; Pau B; Juan J M; Bali J P; de la Coussaye J E; Dauzat M. (Laboratory of Cardiovascular Physiology, University of Montpellier-Nimes, Nime, France.)CLINICA CHIMICA ACTA, (1999 May) 283 (1-2) 43-56. Journal code: DCC. ISSN: 0009-8981. Pub. country: Netherlands. Language: English.

AB The study was designed to determine the time-course of cardiac **troponin I** (cTn-I) release in isolated and Langendorff-perfused rat hearts during **hypoxia** and reoxygenation (H/Reox), and after various durations of total **ischemia** and subsequent **reperfusion** (I/R). For this purpose, in H/Reox, cTn-I was measured with the conventional Access immunoassay (ng/ml) and a new immunoassay which operates at pg/ml, and compared with creatine kinase (CK), **lactate dehydrogenase** (LD) and cardiac **troponin T** (cTn-T). In I/R, cTn-I was compared with CK and LD. The anti-Tn-I mAbs used in cTn-I **assays** cross-react with cTn-I of the rat. A clear difference between time-courses and concentration levels of cTn-I in I/R and H/Reox models was found. In I/R, maximum release of cTn-I, CK and LD similarly occurred within minutes following **reperfusion**; however cTn-I did not return to baseline values. cTn-I levels were not linked to the duration of **ischemia**. In I/R, we were only able to detect small cTn-I concentrations. In H/Reox experiments, cTn-I, CK and LD increased time-dependently. We found higher cTn-I maximal peak levels detected with the Access immunoassay than

with the new **assay** (median, 0.346 ng/ml per min/g dry wt vs 132 pg/ml per min/g dry wt). cTn-T maximal concentrations were lower than maximal cTn-I levels (median, 0.117 ng/ml per min/g dry wt). Time-courses of cTn-I release were roughly similar with both **assays** in the H/Reox model (r = 0.90). These data indicate that the cTn-I time-course is related to experimental model (I/R or H/Reox), but also likely depends on the sensitivity of cTn-I **assays** in such experimental conditions.

L46 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2000 ACS
1998:794450 Document No. 130:249019 Diagnostic value of the determination of cardiac **troponin T** to early myocardial injury. Liang, Shufeng; Yan, Huixia; Xiao, Chuanshi (Shanxi Medical University 2nd Hospital, Taiyuan, 030001, Peop. Rep. China). Shanxi Yiyao Zazhi, 27(5), 410-411 (Chinese) 1998. CODEN: SIYCDB. ISSN: 0253-9926. Publisher: Shanxi Yiyao Zazhi Bianjibu.
AB **ELISA** kit was used to det. serum **troponin T** in 60 patients with myocardial infarction and 60 healthy subjects. Serum **troponin T** was a regulatory protein with relatively small mol. wt. than that of the creatine kinase and **lactate dehydrogenase** that can be released early during the myocardial cell necrosis induced by **ischemia hypoxia**. The 40 cases of healthy subjects serum **troponin T** was 0 and the maximal value was 0.027 .mu.g/L, no correlation was obsd. with the subject age. Taking serum **troponin T** > 0.02 .mu.g/L as the pos. criteria, the diagnostic specificity, accuracy and sensitivity in the 60 patients with myocardial infarction were 100, 97, and 96% resp.; and the pos. prediction value was 96%. Methodol. evaluation obsd. that the av. recovery was 100.9%, intra and inter-**assay** CV were 2.4 and 6.5% resp. The results suggest that the detn. of **troponin T** is highly sensitive and specific for the early diagnosis of myocardial injury.

L46 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 3
1998:165026 Document No. 128:306875 The use of biochemical markers in **ischemic** heart disease: summary of the roundtable and extrapolations. Henderson, A. R.; Gerhardt, W.; Apple, F. S. (University Campus, Department of Biochemistry, London Health Sciences Centre, London, ON, Can.). Clin. Chim. Acta, 272(1), 93-100 (English) 1998. CODEN: CCATAR. ISSN: 0009-8981. Publisher: Elsevier Science B.V..
AB A review, with 27 refs. Acceptable biochem. markers of **ischemic** heart disease are now considered to include myoglobin, CK-MB isoforms, CK-MB, and cardiac **troponins T** and I. AST (SGOT), total LD and LD isoenzymes, and total CK activity measurements are regarded as obsolete for this purpose. All acceptable biochem. markers must be available, if required, with a turnaround time of <20 min. Such a service can either be provided by quant. **assays** in a well-equipped lab. or by qual. point-of-care (bedside) devices (except for the CK-MB isoform **assay**) which can also be used in patients' homes and ambulances. There is, however, a pressing need for the careful side-by-side assessment of the relative merits of each of these biochem. markers to permit definitive conclusions about their future usage. A particular problem is the lack of primary stds. for CK-MB and **troponin I assays**. The sensitivity of the initial ECG is about 50% for detecting myocardial damage; thus the use of

biochem. markers may contribute to the early diagnosis and monitoring of thrombolytic therapy and these possible applications are examd. In addn., biochem. markers are presently the gold std. for the diagnosis of minor myocardial damage. There is now good evidence that biochem. markers, particularly the cardiac troponins, have a prognostic function in **ischemic** heart disease although such findings pose unanswered clin. management questions. At the same time, it is recognized that there is often no need at all for the use of any biochem. marker when the clin. diagnosis is unequivocal, other than for prognosis, monitoring thrombolytic therapy, or diagnosing re-infarction.

L46 ANSWER 7 OF 14 MEDLINE DUPLICATE 4

1998016711 Document Number: 98016711. Cardiac **troponin T** is a sensitive, specific biomarker of cardiac injury in laboratory animals. O'Brien P J; Dameron G W; Beck M L; Kang Y J; Erickson B K; Di Battista T H; Miller K E; Jackson K N; Mittelstadt S. (Human Safety Department, Procter and Gamble Company, Cincinnati, Ohio, USA.)LABORATORY ANIMAL SCIENCE, (1997 Oct) 47 (5) 486-95. Journal code: KYS. ISSN: 0023-6764. Pub. country: United States. Language: English.

AB A reliable serum **assay** that can discriminate between cardiac and skeletal muscle injury is not available for diagnostic use in laboratory animals. We tested and supported the hypotheses that serum cardiac **troponin T** (cTnT) was widely applicable in laboratory animals as a biomarker of cardiac injury arising from various causes; that it increased in proportion to severity of cardiac injury; and that it was more cardiospecific than creatine kinase (CK) or **lactate dehydrogenase** (LD) isozyme activities. In canine and rat models of myocardial infarction, cTnT concentration increased 1,000- to 10,000-fold and was highly correlated with infarct size within 3 h of injury. Serum CK and LD isozymes were substantially less effective biomarkers and, in contrast to cTnT, were ineffective markers in the presence of moderate skeletal muscle injury, with resulting serum CK activity > 5,000 U/L. Using these animal models, and mouse and ferret models, we also showed cTnT to be an effective biomarker in doxorubicin cardiotoxicosis, traumatic injury, **ischemia**, and cardiac puncture. Reference range serum concentrations for all species were at the detection limit of the **assay**, except those for mice, in which they were slightly increased, possibly because mice were used to generate **assay** monoclonal antibodies. We conclude that cTnT is a powerful biomarker in laboratory animals for the sensitive and specific detection of cardiac injury arising from various causes.

L46 ANSWER 8 OF 14 MEDLINE DUPLICATE 5

96103403 Document Number: 96103403. Diagnostic efficiency of **troponin T** measurements in rats with experimental myocardial cell damage. Bleuel H; Deschl U; Bertsch T; Bolz G; Rebel W. (Boehringer Mannheim GmbH, Department of Experimental Toxicology, University of Heidelberg, Germany.)EXPERIMENTAL AND TOXICOLOGIC PATHOLOGY, (1995 May) 47 (2-3) 121-7. Journal code: BIR. ISSN: 0940-2993. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB A cardioselective parameter has been available for about 2 years since the development by KATUS of an immunoassay for cardiac **Troponin T** (TnT). The major advantages of this TnT **assay** are its cardiospecificity and its sensitivity. The parameters usually determined

in toxicity studies in rats to detect alterations in the myocardial cells,
e.g. aspartate aminotransferase (ASAT), creatinine kinase (CK) and **lactate dehydrogenase** (LDH), are either of low sensitivity in this species or give falsely high results as the consequence of stress or haemolysis. We therefore investigated in the present study how well **Troponin T**, determined with the **ELISA Troponin T** from Boehringer Mannheim, can detect experimentally induced myocardial lesions in rats. In order to achieve **hypoxic** damage of the cardiomyocytes in these experiments in rats, male Sprague-Dawley rats were given two doses of 4 mg/kg isoprenaline each (Aludrin from Boehringer Ingelheim, FRG) subcutaneously. The second dose was given 7 h after the start of the experiment. Serum samples were analysed for **Troponin T** (TnT) levels and, for comparison, aspartate aminotransferase (ASAT), creatine kinase (CK), and **lactate dehydrogenase** (LDH). Histological examinations of the heart muscle were performed 24 and 96 h after the first injection. As expected, histological examinations of the isoprenaline-treated animals revealed marked myofibrillic degeneration of the myocardium 24 h after the first injection. Markedly elevated serum TnT levels (up to 7.9 ng/ml) were already evident in these animals after 6 h. TnT values decreased with time, but were still statistically significant after 48 h. Of the well-established indicators for diagnosing myocardial infarction, only ASAT showed transient statistically significant increases over 24 h.(ABSTRACT TRUNCATED AT 250 WORDS)

L46 ANSWER 9 OF 14 MEDLINE DUPLICATE 6
95236625 Document Number: 95236625. Biochemical markers of myocardial damage. Bhayana V; Henderson A R. (Department of Laboratory Medicine, University Hospital (University of Western Ontario), London, Canada..)CLINICAL BIOCHEMISTRY, (1995 Feb) 28 (1) 1-29. Ref: 208. Journal code: DBV. ISSN: 0009-9120. Pub. country: United States. Language: English.
AB OBJECTIVE: To assess various biochemical markers of myocardial damage. METHODS AND RESULTS: Before routinely using any test as a biochemical marker of myocardial damage, the published evidence for its diagnostic utility must be critically assessed. Such assessment includes receiver operator curve (ROC) curve analyses, confidence interval estimates of claimed sensitivity and specificity values, and the effects of testing in serial and parallel modes. It is also necessary to establish the test's rule-in (high specificity) and rule-out (high sensitivity) decision thresholds that may vary with time after the onset of symptoms. The spectrum of **ischemic** heart disease includes acute (sudden death, non-Q- and Q-wave infarctions) and chronic (stable, unstable, and variant angina) conditions. Biochemical markers of myocardial damage are of most value in the diagnosis of acute **ischemic** heart disease, although increasingly some of these markers are being found to possess a prognostic value in chronic **ischemic** heart disease. The markers of enzymatic activity include aspartate aminotransferase, creatine kinase (together with isoenzymes and isoforms), and **lactate dehydrogenase** and isoenzymes. Creatine kinase isoenzyme-2 may also be measured immunologically, and this type of **assay** is in increasing use both because of its speed and because its blood levels rise earlier than the corresponding activities. The commercially available nonenzymatic markers are myoglobin and **troponin T**; **troponin I** is expected to become available in late 1995. While myoglobin is a nonspecific indicator of myocardial damage, its diagnostic value is due to its early appearance in blood. **Troponin**

T is more cardiac specific, but the published data appears to suggest that the cardiac specificity of **troponin I** is superior. Troponin levels become abnormal at about the same time after the onset of symptoms as mass **assays** of creatine kinase isoenzyme-2; therefore, they are not useful as early markers of myocardial damage. CONCLUSION: The availability of these nonenzymatic markers of myocardial damage must force a reassessment of the continued use of the enzymatic markers. Are they necessary, and if so, which ones should be retained?

L46 ANSWER 10 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

94034565 EMBASE Document No.: 1994034565. Cardiac **troponin T** and CK-MB mass release after visually successful percutaneous transluminal coronary angioplasty in stable angina pactoris. Ravkilde J.; Nissen H.; Mickley H.; Andersen P.E.; Thayssen P.; Horder M.. Department of Medicine-Cardiology A, Aarhus University Hospital, Aarhus Amtssygehus, Tage Hansensgade 2,DK-8000 Aarhus C, Denmark. American Heart Journal 127/1 (13-20) 1994.

ISSN: 0002-8703. CODEN: AHJOA2. Pub. Country: United States. Language: English. Summary Language: English.

AB The incidence of cardiac **troponin T** (Tn-T) and creatine kinase (CK) isoenzyme MB mass release was studied in 23 patients with stable angina pectoris undergoing visually successful percutaneous transluminal coronary angioplasty (PTCA). Serial blood samples were drawn for measurement of serum Tn-T, CK-MB mass, total CK activity, CK-MB activity, and **lactate dehydrogenase** isoenzyme (LD-1). ST segment monitoring was carried out during PTCA and for the following 24 hours. None of the patients showed electrocardiographic (ECG) evidence of myocardial infarction. However, Tn-T was elevated in three patients (0.23 to 1.32 .mu.g/L), and in these three and an additional three patients CK-MB mass was also elevated (7.0 to 27.5 .mu.g/L). Total CK activity and LD-1 were only elevated in one of these six patients. None had elevated CK-MB activity. ST segment depression on ECG recording was not predictive of Tn-T or CK-MB mass release. Patients with elevated Tn-T or CK-MB mass did not differ with respect to demographic data, stenosis characteristics, or in the PTCA procedure. We conclude that CK-MB mass uncovers clinically and ambulatory electrocardiographically inapparent severe myocardial **ischemia**/minor myocardial damage (microembolization) in 26% (6 of 23) of patients after visually successful PTCA; 13% (3 of 23) had elevated Tn-T, indicating minor myocardial damage. The application of these markers in the future could be of considerable value for determining the efficacy of coronary angioplasty and atherectomy, as well as for drug therapy in connection with such procedures.

L46 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2000 ACS

1992:548347 Document No. 117:148347 Evaluation of serum **troponin T** measurement in acute myocardial infarction. Uji, Yoshinori; Sugiuchi, Hiroyuki; Okabe, Hiroaki (Med. Sch., Kumamoto Univ., Kumamoto, 860, Japan). Rinsho Byori, 40(7), 775-82 (Japanese) 1992. CODEN: RBYOAI.

ISSN: 0047-1860.

AB A monoclonal solid phase EIA has been developed for the detection of human **troponin T** (I). The serum I levels in healthy subjects were 0.05 .+-. 0.06 ng/mL in total (n = 176), 0.06 .+-. 0.07 ng/mL in males (n = 79) and 0.03 .+-. 0.0 ng/mL in females. Within-run and between-run precision (CVs) of the **assay** were <5%. Various

common interfering factors tested did not affect on the **assay**, but increased titers of rheumatoid factor and anti-coagulants such as EDTA, heparin, oxalate and citrate affected the **assay**. In all patients with defined acute myocardial infarction, serum I levels increased 7- to 10-fold the upper ref. range within 6 h after the onset of chest pain and max. elevation of serum I level was at round 20 h and its levels remained elevated for 7 to 20 days. Specificity and sensitivity for acute myocardial infarction was 92.4% and 100%, resp. The results indicate that I measurement improved the diagnostic efficiency for the detection of myocardial necrosis as compared with conventionally used cardiac enzymes and was an effective tool for the confirmation of the **reperfusion** by PTCA (percutaneous transluminal coronary angioplasty) and PTCR (percutaneous transluminal coronary recanalization).

L46 ANSWER 12 OF 14 BIOSIS COPYRIGHT 2000 BIOSIS
1992:489566 Document No.: BR43:98766. **TROPONIN T** IN CORONARY EFFLUENT FROM ISOLATED RAT HEARTS DURING **HYPOXIA** AND REOXYGENATION. YAMAHARA Y; ASAYAMA J; OHTA B; MATSUMOTO T; MIYAZAKI H; SAKAI R; INOUE M; OMORI I; INOUE D; ; NAKAGAWA M. 2ND DEP. MED., KYOTO PREFECTURAL UNIV. MED., JPN.. XIVTH CONGRESS OF THE EUROPEAN SOCIETY OF CARDIOLOGY, BARCELONA, SPAIN, AUGUST 30-SEPTEMBER 2, 1992. EUR HEART J. (1992) 13 (ABSTR SUPPL), 437. CODEN: EHJODF. ISSN: 0195-668X. Language: English.

L46 ANSWER 13 OF 14 MEDLINE DUPLICATE 7
93098764 Document Number: 93098764. Release kinetics of cardiac **troponin T** in coronary effluent from isolated rat hearts during **hypoxia** and reoxygenation. Asayama J; Yamahara Y; Ohta B; Miyazaki H; Tatsumi T; Matsumoto T; Inoue D; Nakagawa M. (Second Department of Medicine, Kyoto Prefectural University of Medicine, Japan.)BASIC RESEARCH IN CARDIOLOGY, (1992 Sep-Oct) 87 (5) 428-36. Journal code: 9K3. ISSN: 0300-8428. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB A newly developed **troponin T** (TnT) test for the detection of myocardial cell necrosis has been reported to be very efficient in the detection of acute myocardial infarction. The aim of the present study was to determine whether cardiac TnT in coronary effluent from isolated heart perfused with albumin-free perfusion medium could be detected using the enzyme-linked immuno-sorbent **assay** developed by Katus et al. Isolated rat hearts were perfused according to the method of Langendorff. Coronary flow rate was measured by timed collection of the coronary perfusate that dripped from the hearts during 5 h of **hypoxia** (protocol A) or 4 h of **hypoxia** followed by 1 h of reoxygenation (protocol B). Creatine kinase (CK) and **lactate dehydrogenase** (LD) levels were compared with that of TnT. Myocardial adenine nucleotides were measured by **HPLC**. There was a strong correlation between TnT levels in albumin-free coronary effluent and TnT levels in coronary effluent diluted 1:1 with 5% bovine serum albumin ($r = 0.996$, $N = 72$). The coefficients of correlation between TnT and CK or LD during **hypoxia** and reoxygenation were 0.891 ($N = 88$) and 0.871 ($N = 88$), respectively. The coefficient of correlation between CK and LD was 0.993 ($N = 88$). There were no significant differences in either the decrease of coronary flow or the increase of TnT content between the hearts in the two protocols. There was no significant correlation between sigma TnT and energy charge of adenine nucleotides. These results indicate that cardiac TnT levels can be easily measured in albumin-free coronary effluent of isolated heart preparations.(ABSTRACT

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L46 ANSWER 14 OF 14 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 8
1992:451734 Document No.: BA94:93134. CARDIAC **TROPONIN T**
IN THE DIAGNOSIS OF MYOCARDIAL INJURY. MAIR J; DIENSTL F; PUSCHENDORF B. INSTITUT FUER MEDIZINISCHE CHEMIE UND BIOCHEMIE, UNIVERSITAET INNSBRUCK, FRITZ-PREGLSTRASSE 3, A-6020 INNSBRUCK, AUSTRIA.. CRIT REV CLIN LAB SCI, (1992) 29 (1), 31-57. CODEN: CRCLBH. ISSN: 0590-8191. Language: English.

AB In the last several decades serum levels of cardiac enzymes and isoenzymes have become the final arbiters by which myocardial damage is diagnosed or excluded. Because conventionally used enzymes are neither perfectly sensitive nor specific, there is need for a new sensitive and cardiospecific marker of myocardial damage. Cardiac **troponin T** (TnT) is a contractile protein unique to cardiac muscle and can be differentiated by immunologic methods from its skeletal-muscle isoform.

An enzyme immunoassay specific for cardiac TnT is now available in a commercial kit for routine use. The biggest advantage of this **assay** is its cardiospecificity. TnT measurements, however, are also highly sensitive in diagnosis of mycoardial injury and accurately discern even small amounts of myocardial necrosis. TnT measurements are, therefore, particularly useful in patients with borderline CK-MB and in clinical settings in which traditional enzymes fail to diagnose myocardial damage efficiently because of lack of specificity - for example, perioperative myocardial infarction or blunt heart trauma. TnT release kinetics reveal characteristics of both soluble, cytoplasmic, and structurally bound molecules. It starts to increase a few hours after the onset of myocardial damage and remains increased for several days. TnT allows late diagnosis of myocardial infarction. The diagnostic efficiency remains at 98% until 6 d after the onset of infarct-related symptoms. TnT is also useful in monitoring the effectiveness of thrombolytic therapy in myocardial infarction patients. The ratio of peak TnT concentration on day 1 to TnT concentration at day 4 discriminates between patients with successful (> 1) and failed (.ltoreq. 1) **reperfusion**. TnT measurements are very sensitive and specific for the early and late diagnosis of myocardial damage and could, therefore, provide a new criterion in laboratory diagnosis of the occurrence of myocardial damage.

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L53 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2000 ACS
1999:444526 Document No. 131:56156 Methods of diagnosing muscle damage by studying **myofilament protein modification** products. Van Eyk, Jennifer E.; Iscoe, Steven D.; Simpson, Jeremy A. (Queen's University at Kingston, Can.). Can. Pat. Appl. CA 2243372 AA 19990116, 77 pp. (English). CODEN: CPXXEB. APPLICATION: CA 1998-2243372 19980716. PRIORITY: US 1997-52697 19970716; US 1998-115589 19980715.

AB A method for assessing muscle damage in a biol. sample obtained from a subject is disclosed. The method involves obtaining a biol. sample from a subject being assessed for muscle damage, and evaluating the sample for the presence or absence of a **myofilament protein modification** product. The method can also be used to assess the extent and/or type of muscle damage in a subject by studying the profile of **myofilament protein modification** products detected in the sample taken from the subject. The invention further provides a method for screening for an agent which modulates the level of a **myofilament protein modification** product present in a biol. sample or for a calcium sensitizing agent. The invention is applicable to cardiac muscle and skeletal muscle. SDS-PAGE and Western blot anal. of rat cardiac muscle tissue samples indicated that the extent and type of modification to **troponin I** changed depending on whether mild or severe **ischemic** damage had occurred.

L53 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2000 ACS
1999:701153 **Hypoxic** response of synaptosomal proteins in term guinea pig fetuses. Buonocore, Giuseppe; Liberatori, Sabrina; Bini, Luca; Mishra, Om P.; Delivoria-Papadopoulos, Maria; Pallini, Vitaliano; Bracci, Rodolfo (Institute of Preventive Pediatrics and Neonatology, University of Siena, Siena, 53100, Italy). J. Neurochem., 73(5), 2139-2148 (English) 1999. CODEN: JONRA9. ISSN: 0022-3042. Publisher: Lippincott Williams & Wilkins.

AB Early events in the **hypoxia**-induced response trigger tyrosine phosphorylation cascades involving a large no. of enzymes and substrates. The resolving power of advanced two-dimensional gel electrophoresis, followed by **immunoblotting** with specific antibodies to phosphotyrosine, has been used to analyze **hypoxia**-induced modifications in guinea pig brain synaptosomes. These procedures, in conjunction with computer-aided image anal., are useful in the differential display of gene products, providing comparison at the level of post-translationally modified products. Studies were performed in cerebral cortical synaptosomes from three normoxic and three **hypoxic** newborn guinea pigs. To filter off background noise consisting of non-reproducible migrating protein spots, only reproducible features of electrophoretic patterns were considered. Immunoreactivity patterns obtained with anti-phosphotyrosine antibodies proved to be different in normoxic and **hypoxic** synaptosomes: of a total of 130 immunoreactive spots, 49 were tyrosine-phosphorylated in **hypoxic** synaptosomes only and 20 in the normoxic ones only. Our data suggest that **hypoxia** extensively remodels the signaling pathway by switching off tyrosine phosphorylation of some cellular components (i.e., .alpha.-internexin) and switching on tyrosine phosphorylation of some other proteins (i.e., heat shock cognate 70, aconitase, 2',3'-cyclic nucleotide 3'-phosphodiesterase, and pyruvate kinase).

L53 ANSWER 3 OF 4 MEDLINE DUPLICATE 1
95295012 Document Number: 95295012. Intracellular compartmentation of **troponin T**: release kinetics after global **ischemia** and calcium paradox in the isolated perfused rat heart. Remppis A; Scheffold T; Greten J; Haass M; Greten T; Kubler W; Katus H A. (University of Heidelberg, Germany.)JOURNAL OF MOLECULAR AND CELLULAR CARDIOLOGY, (1995 Feb) 27 (2) 793-803. Journal code: J72. ISSN: 0022-2828. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The marked differences in **troponin T** serum concentrations observed in patients with **reperfused** and non-**reperfused** myocardial infarction may be due to a perfusion dependent wash-out of an unbound fraction of cardiac **troponin T**. To test the release kinetics of **troponin T** experimentally, the isolated rat heart (Langendorff preparation) was damaged either by the calcium paradox or by no-flow **ischemia**. Following membrane damage by the calcium paradox **troponin T** (TNT) showed the same release kinetics in the coronary effluent as the cytosolic markers creatine kinase (CK) or **lactate dehydrogenase** (LDH). Peak levels of **troponin T** (282 +/- 58 micrograms/l), CK (6754 +/- 1642 U/l), and LDH (5817 +/- 1730 U/l) occurred 5 min after onset of **reperfusion** with calcium containing buffers and returned to 9.9%, 1.3%, and 1% of their respective peak levels within 55 min of **reperfusion**. During **reperfusion** after no-flow **ischemia** different release kinetics were found for cytosolic enzymes and **troponin T**. After 60 min of **ischemia**, **troponin T** levels in the coronary effluent increased over the entire **reperfusion** period of 55 min, almost doubling the 5 min value (191%). In contrast, cardiac enzymes rapidly declined to 18% (CK) and 23% (LDH) of their respective 5 min values at the end of **reperfusion**. Light microscopy after **reperfusion** with carbon black revealed a complete and homogeneous **reperfusion** of Langendorff hearts after no-flow **ischemia**. **Immunoblot** analysis confirmed the release of an undegraded 39 kDa **troponin T** molecule, both after global **ischemia** and the calcium paradox. These data indicate that prolonged **ischemia** induces a continuous liberation of cardiac **troponin T**, most probably from disintegrating myofibres, whereas membrane damage leads almost exclusively to leakage of a functionally unbound **troponin T** pool. These findings may explain the biphasic serum concentration changes of cardiac **troponin T** in patients with **reperfused** myocardial infarction.

L53 ANSWER 4 OF 4 MEDLINE
92233571 Document Number: 92233571. Reoxygenation of endothelial cells increases permeability by oxidant-dependent mechanisms. Lum H; Barr D A; Shaffer J R; Gordon R J; Ezrin A M; Malik A B. (Department of Physiology and Cell Biology, Albany Medical College of Union University, Albany, NY 12208..)CIRCULATION RESEARCH, (1992 May) 70 (5) 991-8. Journal code: DAJ. ISSN: 0009-7330. Pub. country: United States. Language: English.

AB We investigated the effects of **hypoxia**/reoxygenation exposure on the barrier function of endothelial cell monolayers. Bovine pulmonary microvessel endothelial cells were grown to confluence on microporous filters (0.8-microns pore diameter) and exposed to **hypoxia** (0.1% O2 or PO2 approximately 1 mm Hg) for 2, 4, 12, or 24 hours, followed by reoxygenation with room air for a period ranging from 16 seconds to 2 hours. The transendothelial clearance rate of 125I-albumin was measured to determine the permeability of endothelial monolayers. Permeability increased twofold or fivefold over control values after 1 hour of

reoxygenation in monolayers that had been exposed to either 12 or 24 hours of **hypoxia**. The response occurred within 5 minutes of reoxygenation, increased maximally by 40 minutes, and remained elevated with continuous reoxygenation for up to 2 hours. The increase in permeability was associated with F-**actin** reorganization, a change to spindlelike cells, and injured mitochondria. **Immunoblot** analysis indicated that neither **hypoxia** alone nor reoxygenation changed CuZn superoxide dismutase (SOD), MnSOD, and catalase levels. However, release of superoxide anions (O2-) into the extracellular medium increased by twofold within 40-60 minutes of reoxygenation. Treatment of endothelial cells with CuZnSOD (100 units/ml) for the 24-hour **hypoxia** period prevented O2- generation and approximately 50% of the increase in permeability. Higher CuZnSOD concentrations (greater than or equal to 200 units/ml) were not protective. Treatment with catalase (100-1,000 units/ml) inhibited the reoxygenation-induced increase in permeability at the highest catalase concentration (1,000 units/ml), suggesting a critical role of hydrogen peroxide in mediating the response.(ABSTRACT TRUNCATED AT 250 WORDS)

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L61           1 FILE CAPLUS
L62           5 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L63           1 FILE EMBASE
L64           0 FILE WPIDS

TOTAL FOR ALL FILES
L65           8 LABUGGER R?/AU,IN


'IN' IS NOT A VALID FIELD CODE
L66           0 FILE MEDLINE
L67           0 FILE CAPLUS
L68           0 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L69           0 FILE EMBASE
L70           0 FILE WPIDS

TOTAL FOR ALL FILES
L71           0 NEYEROVA I?/AU,IN

=> s l65 and l59

L72           0 FILE MEDLINE
L73           0 FILE CAPLUS
```

L74 0 FILE BIOSIS
L75 0 FILE EMBASE
L76 0 FILE WPIDS

TOTAL FOR ALL FILES
L77 0 L65 AND L59

=> s (165 or 159) and muscle

L78 1 FILE MEDLINE
L79 0 FILE CAPLUS
L80 0 FILE BIOSIS
L81 0 FILE EMBASE
L82 0 FILE WPIDS

TOTAL FOR ALL FILES
L83 1 (L65 OR L59) AND MUSCLE

=> d cbib abs

Applicant

L83 ANSWER 1 OF 1 MEDLINE
96207482 Document Number: 96207482. Recombinant troponin I substitution and calcium responsiveness in skinned cardiac **muscle**. Strauss J D; **Eyk J E**; Barth Z; Wiesner R J; Ruegg J C; **Eyk J E**; Kluwe L; Kluwe L. (Department of Physiology II, University of Heidelberg, Im Neuenheimer Feld 326, D-69120 Heidelberg, Germany.)PFLUGERS ARCHIV. EUROPEAN JOURNAL OF PHYSIOLOGY, (1996 Apr) 431 (6) 853-62. Journal code: OZX. ISSN: 0031-6768. Pub. country: GERMANY: Germany, Federal Republic of.
Language: English.
AB Using treatment with vanadate solutions, we extracted native cardiac troponin I and troponin C (cTnI and cTnC) from skinned fibers of porcine right ventricles. These proteins were replaced by exogenously supplied TnI and TnC isoforms, thereby restoring Ca2+-dependent regulation. Force then depended on the negative logarithm of Ca2+ concentration (pCa) in a sigmoidal manner, the pCa for 50% force development, pCa50, being about 5.5. For reconstitution we used fast-twitch rabbit skeletal **muscle** TnI and TnC (sTnI and sTnC), bovine cTnI and cTnC or recombinant sTnIs that were altered by site-directed mutagenesis. Incubation with TnI inhibited isometric tension in TnI-extracted fibers in the absence of Ca2+, but restoration of Ca2+ dependence required incubation with both TnI and TnC. Relaxation at low Ca2+ levels and the steepness of the force/pCa relation depended on the concentration of exogenously supplied TnI in the reconstitution solution (range 20-150 "mu"M), while Ca2+ sensitivity, i.e. the pCa50, was dependent on the isoform, and also on the concentration of TnC in the reconstitution solution. At pH 6.7, skinned fibers reconstituted with optimal concentrations of sTnC and sTnI (120 "mu"M and 150 "mu"M, respectively) were more sensitive to Ca2+ than those reconstituted with cTnC and cTnI (difference in pCa50 approx. 0.2 units). Rabbit sTnI was cloned and expressed in Escherichia coli using a high yield expression plasmid. We introduced point mutations into the TnI inhibitory region comprising the sequence of the minimal common TnC/actin binding site (-G104-K-F-K-R-P-P-L-R-R-V-R115-). The four mutants produced by substitution of T for P110, G for P110, G for L111, and G for K105 were chosen, based on previous work with synthetic peptides showing that single